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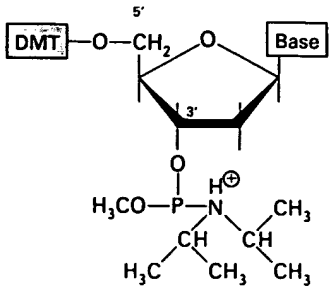
tire *E. coli* genome (3×10^6 base pairs) has now become feasible. We can even begin to think about determining the sequence of extensive stretches of the human genome, which contains 3×10^9 base pairs.

DNA PROBES AND GENES CAN BE SYNTHESIZED BY AUTOMATED SOLID-PHASE METHODS

DNA strands, like polypeptides (p. 66), can be synthesized by the sequential addition of activated monomers to a growing chain that is linked to an insoluble support. The activated monomers are protonated *deoxyribonucleoside 3′-phosphoramidites* (Figure 6-10). In step 1, the



Protonated phosphoramidite
(The 5′-hydroxyl is blocked by a dimethoxytrityl protecting group.)

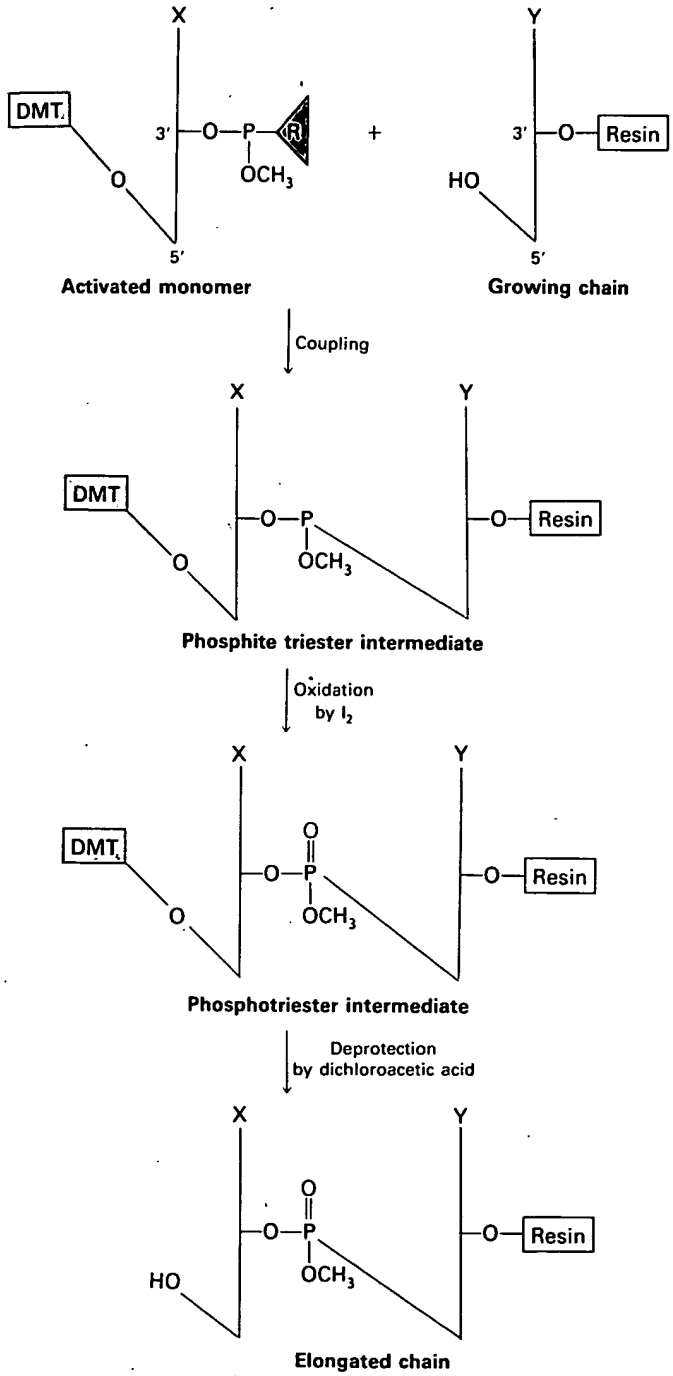
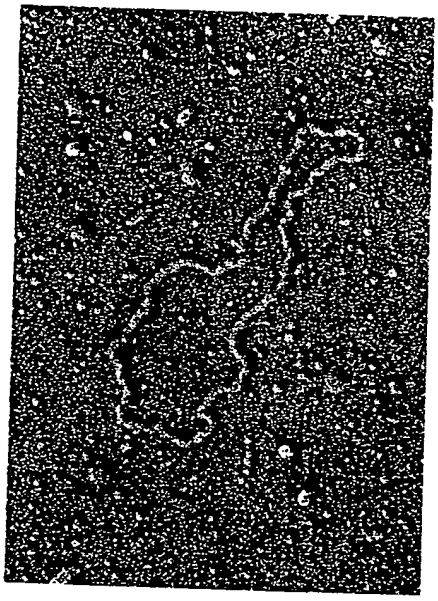


Figure 6-10
Solid-phase synthesis of a DNA chain by the phosphite triester method. The activated monomer added to the growing chain is a deoxyribonucleoside 3′-phosphoramidite containing a DMT (dimethoxytrityl) blocking group on its 5′-oxygen atom.



Electron micrograph of pSC101, the first plasmid vector used in the cloning of DNA. [Courtesy of Dr. Stanley N. Cohen.]

3′-phosphorus atom of this incoming unit becomes joined to 5′-oxygen of the growing chain to form a *phosphite triester*. The 5′ of the activated monomer is unreactive because it is blocked by dimethoxytrityl (DMT) protecting group. Likewise, amino groups the purine and pyrimidine bases are blocked. Coupling is carried under anhydrous conditions because water reacts with phoramidites. In step 2, the phosphite triester (in which P is trivalent) oxidized by iodine to form a *phosphotriester* (in which P is pentavalent). In step 3, the DMT protecting group on the 5′-OH of the growing chain is removed by addition of dichloroacetic acid, which leaves other protecting groups intact. The DNA chain is now elongated by one unit and ready for another cycle of addition. Each monomer addition cycle takes only about ten minutes and elongates more than 98% of the chains.

This solid-phase approach is ideal for the synthesis of DNA, as it is for polypeptides, because the desired product stays on the insoluble support until the final release step. All of the reactions occur in a single vessel, and excess soluble reagents can be added to drive reactions to completion. At the end of each step, soluble reagents and by-products are washed away from the glass beads that bear the growing chains.

After assembly of the desired DNA chain, the methyl groups protecting the phosphates are removed by addition of thiophenol. The DNA strand is then released from the glass bead by cleavage of the ester bond between the 3′-OH of the terminal nucleoside and the resin that links it to the glass support. This bond is hydrolyzed by the addition of concentrated ammonium hydroxide. Finally, the benzoyl and isobutyryl groups protecting the bases are removed by heating the DNA in ammonium hydroxide. Because elongation is never 100% complete, the final DNA chains are of diverse lengths—the desired chain is the longest one. The sample can be purified by high-performance liquid chromatography or by electrophoresis on polyacrylamide gels. DNA chains up to 100 nucleotides long can readily be synthesized by this automated method.

The ability to rapidly synthesize DNA chains of any selected sequence opens many experimental avenues. For example, an oligonucleotide labeled at one end with ^{32}P can be used to search for a complementary sequence in a very long DNA molecule or even in a genome consisting of many chromosomes. The use of labeled oligonucleotides as *DNA probes* is powerful and general. For example, a DNA probe that is base-paired to a known complementary sequence in a chromosome can serve as the starting point of an exploration of adjacent uncharted DNA. For example, the probe can be used as a *primer* to initiate the replication of neighboring DNA by DNA polymerase. One of the most exciting applications of the solid-phase approach is the *synthesis of new tailor-made genes*. New proteins with novel properties can now be produced in abundance by expressing synthetic genes. *Protein engineering* has become a reality. Moreover, regulatory sequences in DNA can be changed at will to control gene expression.

NEW GENOMES CAN BE CONSTRUCTED, CLONED, AND EXPRESSED

The pioneering work of Paul Berg, Herbert Boyer, and Stanley Cohen in the early 1970s led to the development of recombinant DNA technology, which has revolutionized biochemistry. New combinations of unre